Marked-up Version of Amendments

I. Amendment to the title:

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Methods And Apparatus For Analyzing Polynucleotide Sequences

II. Amendments to the claims (unamended claims are reproduced in small font)

1. (Amended) A method of analyzing a target polynucleotide comprising:

(a) providing a primed target polynucleotide attached to a microfabricated multilayer elastomeric synthesis channel;

(b) flowing a first nucleotide through the synthesis channel under conditions whereby the first nucleotide attaches to the primer, if a complementary nucleotide is present to serve as template in the target polynucleotide;

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(c) determining presence or absence of a signal, the presence of a signal indicating that the first nucleotide was incorporated into the primer, and hence the identity of the complementary base that served as a template in the target polynucleotide;

(d) removing or reducing the signal, if present;

(e) repeating steps (b)-(d) with a further nucleotide, the same or different from the first nucleotide, whereby the further nucleotide attaches to the primer or a nucleotide previously incorporated into the primer, and

(f) repeating step (e) until identities of the bases in a portion or all of the target polynucleotide are determined.

2. The method of claim 1, wherein

step (a) comprises providing a plurality of different primed target polynucleotides attached to different synthesis channels;

step (b) comprises flowing the first nucleotide through each of the synthesis channels; and

step (c) comprises determining presence or absence of a signal in each of the channels, the presence of a signal in a synthesis channel indicating the first nucleotide was incorporated into the primer in the synthesis channel, and hence the identity of the complementary base that served as a template in the target polynucleotide in the synthesis channel.

3. The method of claim 2, wherein step (a) comprising providing a plurality of different primed target polynucleotides attached to each synthesis channel.

4. The method of claim 1, wherein said first nucleotide and said further nucleotide are labeled.

5. The method of claim 1, further comprising flushing the synthesis channel to remove unincorporated first or further labeled nucleotide.

6. The method of claim 4, wherein steps (b)-(d) are performed at least four times with four different types of labeled nucleotides.

7. The method of claim 4, wherein steps (b)-(d) are performed until the identity of each base in the target polynucleotide has been identified.

8. The method of claim 4, wherein said synthesis channel is formed by bonding a microfluidic chip to a flat substrate.

9. The method of claim 8, wherein said target polynucleotide is immobilized to the interior surface of said substrate in said synthesis channel.

10. The method of claim 9, wherein said interior surface is coated with a polyelectrolyte multilayer (PEM).

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11. (Amended) The method of claim 8, wherein said microfluidic chip is fabricated with an elastomeric material.

12. The method of claim 11, wherein said an elastomeric material is RTV silicone.

13. The method of claim 4, wherein at least one of the labeled nucleotide comprises a mixture of labeled and unlabeled forms of the nucleotide.

14. The method of claim 4, wherein cross section of said synthesis channel has a linear dimension of less than 100 μm x 100 μm, less than 10 μm x 100 μm, less than 1 μm x 10 μm, or less than 0.1 μm x 10 μm.

15. The method of claim 4, wherein said label is a fluorescent label.

16. The method of claim 15, wherein said removing or reducing is by photobleaching.

17. The method of claim 4, wherein said label is a radiolabel.

18. The method of claim 17, wherein said removing or reducing is by chemical or enzymatic release of the label.

19. The method of claim 4, wherein said label is a mass-spectrometric label.

20. The method of claim 19, wherein said removing or reducing is by chemical or enzymatic release of the label.

21. The method of claim 1, wherein said signal is a non-optical signal.

22. The method of claim 21, wherein said non-optical signal is pyrophosphate release.

23. The method of claim 22, wherein said pyrophosphate release is detected with mass spectrometry.

24. The method of claim 22, wherein said pyrophosphate release is detected with an enzymatic reaction.

25. The method of claim 24, wherein said enzymatic reaction is a redox enzymatic reaction.

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26. (Amended) A method of analyzing a target polynucleotide comprising::

(a) pretreating the surface of a substrate with a polyelectrolyte multilayer (PEM) to create surface chemistry that facilitates polynucleotide attachment and sequence analysis;

(b) providing a primed target polynucleotide attached to a surface of a substrate;

(c) providing a labeled first nucleotides to the attached target polynucleotide under conditions whereby the labeled first nucleotide attaches to the primer, if a complementary nucleotide is present to serve as template in the target polynucleotide;

(d) determining presence or absence of a signal, the presence of a signal indicating that the labeled first nucleotide was incorporated into the primer, and hence the identity of the complementary base that served as a template in the target polynucleotide;

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(e) repeating steps (c)-(d) with a labeled further nucleotide, the same or different from the first labeled nucleotide, whereby the labeled further nucleotide attaches to the primer or a nucleotide previously incorporated into the primer, and

(f) repeating step (e) until identities of the bases in a portion or all of the target polynucleotide are determined..

27. The method of claim 26, wherein said substrate is glass and said surface is coated with a polyelectrolyte multilayer (PEM).

28. The method of claim 27, wherein said PEM is terminated with a polyanion.

29. The method of claim 28, wherein said polyanion bears pendant carboxylic acid groups.

30. The method of claim 26, wherein said target polynucleotide is biotinylated, and said surface is coated with streptavidin.

31. The method of claim 30, wherein said surface is coated with biotin prior to coating with streptavidin.

32. The method of claim 31, wherein said surface is coated with a polyelectrolyte multilayer (PEM) terminated with carboxylic acid groups prior to attachment of biotin.

33. The method of claim 32, wherein said surface is pretreated with RCA solution prior to coating with said PEM.

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34. (Amended) A method of analyzing a target polynucleotide comprising:

(a) providing a primed target polynucleotide in a microfabricated multilayer elastomeric synthesis channel;

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(b) providing a first nucleotide under conditions whereby the first nucleotide attaches to the primer, if a complementary nucleotide is present to serve as template in the target polynucleotide; wherein a percentage of molecules of said first nucleotide is labeled.

(c) determining presence or absence of a signal from the primer, the presence of a signal indicating the first nucleotide was incorporated into the primer, and hence the identity of the complementary base that served as a template in the target polynucleotide;

(d) repeating steps (b)-(c) with a further nucleotide, the same or different from the first nucleotide, whereby the further nucleotide attaches to the primer or a nucleotide previously incorporated into the primer; wherein a percentage of molecules of said further nucleotide is labeled, and

(e) repeating step (d) until identities of the bases in a portion or all of the target polynucleotide are determined..

35. The method of claim 34, wherein said label is a fluorescent label.

36. The method of claim 35, wherein said removing or reducing is by photobleaching.

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37. (Amended) The method of claim 36, wherein said percentage of the first nucleotide and said percentage of the further nucleotide are less than 10%.

38. (Amended) The method of claim 37, wherein said percentage of the first nucleotide and said percentage of the further nucleotide are less than 1%.

39. (Amended) The method of claim 38, wherein said percentage of the first nucleotide and said percentage of the further nucleotide are less than 0.1%.

40. (Amended) The method of claim 34, wherein said percentage of the first nucleotide and said percentage of the further nucleotide are less than 0.01%.